



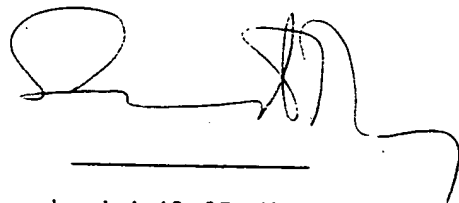
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Verified English-Language Translation of U.S. Provisional
Application Serial No. 60/349,994

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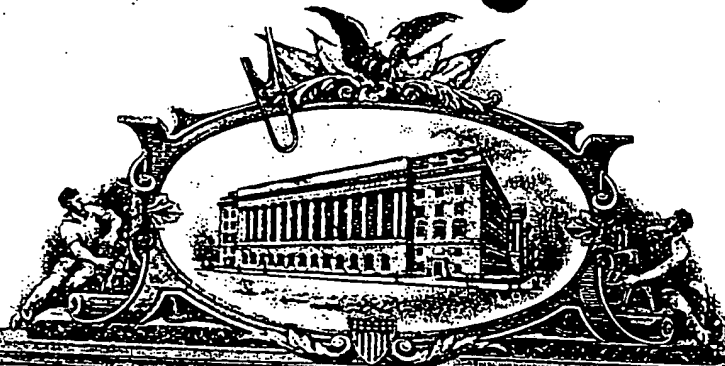
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For and on behalf of RWS Group Ltd

The 22nd day of July 2004



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APPLICATION NUMBER: 60/349,994

FILING DATE: *January 23, 2002*



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L. EDELEN
Certifying Officer

DISINFECTANT/ALCOHOL AMINE II

Description

- 5 The invention relates to the use of synergistic disinfectant compositions based on amines and/or quaternary ammonium salts as virucidal agents, in particular towards polioviruses.
- 10 Numerous disinfectant and preservative compositions based on amines and/or quaternary ammonium salts are known. However, in general, in particular at relatively high dilution, these exhibit an unsatisfactory activity towards fungi, for example *Aspergillus niger* and
- 15 viruses (in particular towards highly resistant viruses, for example polioviruses).

It was therefore an object of the present invention to provide disinfectant compositions based on amines

20 and/or quaternary ammonium salts which exhibit good activity towards fungi and viruses even at high dilution.

This object is achieved according to the invention by

25 the use according to Claim 1.

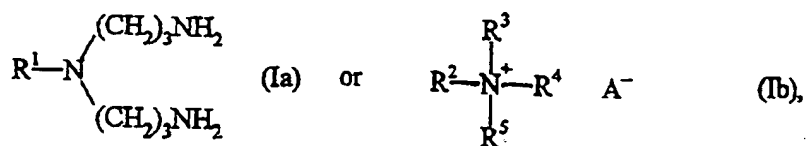
The earlier application PCT/EP 01/10754 describes disinfectant compositions based on amines and/or quaternary ammonium salts which have good effectiveness

30 towards fungi and viruses even at high dilution.

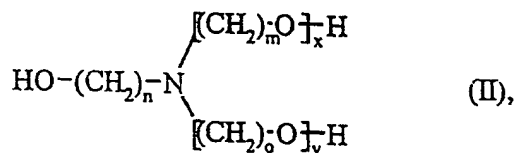
It has now surprisingly been found that such disinfectant compositions also have pronounced virucidal properties and, in particular, good effectiveness towards highly resistant viruses such as

35 polioviruses.

The compositions comprise amines and/or quaternary ammonium salts of the general formula



- where R^1 is C_{6-18} -alkyl
 R^2 is benzyl or C_{6-18} -alkyl
 5 R^3 is C_{1-19} -alkyl or $-\text{[(CH}_2\text{)}_2\text{-O]}_n\text{R}^6$ where $n = 1-20$
 R^4 and R^5 independently of one another are C_{1-4} -alkyl
 R^6 is hydrogen or unsubstituted or substituted phenyl
 and A^- is a monovalent anion or one equivalent of a
 10 polyvalent anion of an inorganic or organic acid;
 and at least one alkanolamine of the general formula



- where n and, if present, m and o independently of one
 15 another have the value 2 or 3
 and x and y independently of one another have the value
 0 or 1, or a corresponding salt; in the mass ratio I:II
 of 20:1 to 1:20.
 20 Alkyl, here and hereinafter, is taken to mean in each
 case unbranched or branched alkyl groups of the
 specified number of carbons, but preferably unbranched
 alkyl groups, and particularly preferably those having
 an even number of carbon atoms. In particular, this is
 25 also taken to mean the homologue mixtures derived from
 natural raw materials, for example "coconutalkyl".

Substituted phenyl is taken to mean, in particular,
 phenyl groups substituted with one or more C_{1-8} -alkyl
 30 groups and/or chlorine atoms.

Suitable anions A^- are in principle all inorganic or
 organic anions, in particular halide, for example

chloride or bromide, or anions of low carboxylic acids, for example acetate, propionate or lactate.

5 The amine or quaternary ammonium salt (Ia/Ib) is preferably *N,N*-bis(3-aminopropyl)dodecylamine, *N,N*-bis-(3-aminopropyl)octylamine, a didecyldimethylammonium salt, dioctyldimethylammonium salt, octyldecyldimethylammonium salt, dicoconutalkyldimethylammonium salt, 10 dicoconutalkylmethylpoly(oxyethyl)ammonium salt, decyldimethylpoly(oxyethyl)ammonium salt, didecylmethylpoly(oxyethyl)ammonium salt, octyldimethylpoly(oxyethyl)ammonium salt, dioctylmethylpoly(oxyethyl)-ammonium salt, coconutalkyldimethylbenzylammonium salt, 15 benzyldodecyldimethylammonium salt or benzyldimethylpoly(oxyethyl)ammonium salt or a mixture of two or more of these compounds. Particularly good results were achieved with didecyldimethylammonium salts.

20 Suitable alkanolamines (II) are in principle all ethanolamines and propanolamines, in particular monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol. Obviously, using mixtures of the said compounds is also within the scope of the 25 invention. Particularly good results have been obtained using the compounds having a primary amino group, that is to say using monoethanolamine and 3-amino-1-propanol.

30 The mass ratio of amine (Ia) or quaternary ammonium salt (Ib) to alkanolamine (II) is preferably in the range from 1:5 to 5:1.

35 The disinfectant compositions used according to the invention preferably comprise water as solvent, if appropriate in combination with an organic solvent.

Preferably, the disinfectant compositions used according to the invention further comprise one or more

aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

- 5 A preferred use of the disinfectant compositions is surface disinfection and instrument disinfection.

Further preferred fields of use are laundry disinfection and hand disinfection.

10

A further preferred use of the disinfectant compositions is the use in chemical toilets, for example on board aircraft and vehicles.

- 15 The examples below illustrate the implementation of the invention, and should not be taken to be a restriction to the embodiments described. All quantities given, where not otherwise specified, are in % by mass. The test microorganism used in each case was *Aspergillus*
20 *niger* ATCC 16404. The effectiveness was determined, unless otherwise specified, using the method specified in CEN 1275.

Example 1

25

A disinfecting cleaner formulation (concentrate) was prepared from:

- 30 5.0% didecyldimethylammonium chloride (50% strength solution)
2.0% N,N-bis(3-aminopropyl)dodecylamine
5.0% monoethanolamine
5.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
35 0.5% sodium metasilicate
0.5% sodium carbonate
2.0% methylglycinediacetic acid trisodium salt (Trilon® M; 40% strength solution)
water to 100%

The effectiveness was determined using a dilution (1 part of concentrate, 99 parts of water) at 20°C and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was 4.1.

Comparative Example 1

The procedure of Example 1 was followed, but with the difference that the monoethanolamine was replaced by the same amount of water. Under the same test conditions, the formulation was virtually inactive.

Example 2

A disinfectant formulation (concentrate) was prepared from:

- 4.9% *N,N*-bis(3-aminopropyl)dodecylamine
- 4.0% monoethanolamine
- 2.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
- 5.0% Hostapur® SAS 30 (C₁₃₋₁₇ secondary *n*-alkanesulphonic acid, sodium salt)
- 2.0% ethylenediaminetetraacetic acid tetrasodium salt (40% strength solution)
- 0.7% ethylenediaminetetraacetic acid
- water to 100%

The effectiveness was determined using a dilution (1 part of concentrate, 199 parts of water) at 20°C and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was 4.3.

Example 3

A disinfectant formulation (concentrate) was prepared from:

- 4.2% *N,N*-bis(3-aminopropyl)dodecylamine
2.0% didecylmethylpoly(oxyethyl)ammonium propionate
(BARDAP 26)
4.0% monoethanolamine
5 2.0% Genapol® T250 (tallow fatty alcohol polyglycol
ether, 25 mol of ethylene oxide)
5.0% Hostapur® SAS 30 (C₁₃₋₁₇ secondary *n*-alkanesulphonic
acid, sodium salt)
2.0% ethylenediaminetetraacetic acid tetrasodium salt
10 (40% strength solution)
0.7% ethylenediaminetetraacetic acid
4.0% butyl diglycol
water to 100%

- 15 The effectiveness was determined using a dilution
(1 part of concentrate, 199 parts of water) at 20°C and
with a contact time of 15 min. The logarithm to base
ten of the reduction in microorganism count was > 4.4.
In addition, the effectiveness was also determined
20 using the method specified in CEN 1650 with a contact
time of 15 min, a concentration of 1.0%, a water
hardness of 30°FH and an organic load of 0.3% albumin.
The logarithm of the reduction in microorganism count
was > 4.4.

25

Examples 4-19

- Aqueous solutions were prepared from 0.5% alkanolamine
(II) and 0.25% of amine or quaternary ammonium salt
30 (Ia/Ib) and tested using the method specified in
CEN 1275. The results are summarized in Table 1.

Table I

Example No.	Amine/ammonium salt	Alkanolamine	log microbial reduction
4	dimethyldioctyl-ammonium chloride	monoethanolamine	4.3
5	ditto	diethanolamine	4.0
6	ditto	triethanolamine	3.6
7	ditto	3-amino-1-propanol	4.2
8	didecyldimethyl-ammonium chloride	monoethanolamine	4.0
9	ditto	diethanolamine	3.8
10	ditto	triethanolamine	3.1
11	ditto	3-amino-1-propanol	4.0
12	di-C ₈₋₁₀ -alkyldimethyl-ammonium chloride (60%)/C ₁₂₋₁₆ -alkyl-benzyl dimethylammonium chloride (40%); Bardac® 205-M	monoethanolamine	3.9
13	ditto	diethanolamine	3.2
14	ditto	triethanolamine	2.8
15	ditto	3-amino-1-propanol	3.8
16	N,N-bis(3-amino-propyl)dodecylamine	monoethanolamine	2.9
17	ditto	diethanolamine	2.7
18	ditto	triethanolamine	2.4
19	ditto	3-amino-1-propanol	2.8

5 For comparison, all compounds listed in Table 1 were tested as individual substances in 0.5% strength solution. None of these compounds exhibited pronounced fungicidal activity (log microbial reduction < 2).

Example 20

A disinfectant formulation (concentrate) was produced from:

- 5 9.9% didecyldimethylammonium chloride (70% strength solution)
- 8.0% monoethanolamine
- 5.0% Genapol®T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
- 10 5.0% potassium carbonate (anhydrous)
- 6.0% ethylenediaminetetraacetic acid tetrasodium salt (Trilon®B; 40% strength solution)
- water to 100%

15

Example 21

The concentrate described in Example 20 was tested in 6% strength dilution in the suspension test using an exposure time of 30, 60 and 120 minutes for effectiveness against poliovirus type 1 (Mahoney strain).

Test method:

- 25 The test was performed in accordance with the "Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren"
- 30 [Guideline of the German Federal Health Agency and the German Association for Controlling Viral Diseases for testing chemical disinfectants for effectiveness against viruses] (*Bundesgesundheitsbl.* 1982, 25, 397).
- The growth medium for the Vero cell cultures was
- 35 "Dulbecco's Modified Eagle's Medium", to which 10% foetal calf serum and 10 U/ml of penicillin and also 10 µg/ml of streptomycin had been added. After the tissue culture was inoculated with poliovirus the tissue culture medium only contained 3% foetal calf

serum. After virtually complete detachment of polio-infected cells, the virus suspension was purified by centrifuging of cells and cell constituents ($3000 \times g$, 15 min). Since the cell culture medium contained 3% foetal calf serum, in the disinfectant test also, a small protein load was also present in the test batches using twice-distilled water.

For the disinfectant test, 1 part of virus suspension was mixed with 8 parts of a 7% strength dilution of the disinfection concentrate (corresponding to a final concentration of 6%) and in each case 1 part of twice-distilled water or 2% strength serum albumin or foetal calf serum and was incubated for 30, 60 and 120 min at 20°C . The activity of the disinfectant was then stopped by 100-fold dilution with cold medium containing no foetal calf serum. In each case 2 wells of multiwell plates containing 6 recesses (Becton Dickinson Labware, Lincoln Park, NJ, Type FalconTM353046) which contained a dense lawn of Vero cells, were inoculated with 1 ml in each case of this dilution (corresponding to a dilution of the virus suspension to 10^{-3}) and further serial 10-fold dilutions. After 1 h of adsorption time at room temperature, the supernatant liquid was drawn off. The cell lawns of the wells were then coated with 2 ml of 2% strength agarose (Serva high EEO, Cat. No. 11397) liquefied by boiling, which had been mixed with twice-concentrated medium containing 5% strength foetal calf serum in a ratio of 1:1, and had been cooled to 40°C in a waterbath. After solidification of the agarose at room temperature, the plates were incubated for 2 days at 37°C in a CO_2 incubation cabinet.

The infectivity of the virus suspension was tested in the plaque test. In this test each area of destroyed cells corresponds to one infectious unit of poliovirus. The number of plaques thus indicates the number of infectious virus particles present in a defined dilution of the test batch. The plaques are visualized by staining 1.0 ml in each case of a solution of 0.1% Brilliant Blue R (Sigma, Cat. No. B0149) for 30 min in

an aqueous solution containing 20% methanol and 5% acetic acid. The unstained plaques are then clearly differentiated from blue-coloured cell lawns. A mean plaque count is calculated from two batches in each case of a dilution.

"Virus controls", in which the starting concentration of the virus was determined, were batches in which the disinfectant had been replaced by the same volume of twice-distilled water. The virus concentration thus determined served as reference for calculating the virus-inactivating action of the disinfectant tested.

"Toxicity controls" for detecting any damage of the tissue culture cells by the disinfectant were batches in which the virus suspension had been replaced by the same volume of twice-distilled water. These batches were diluted in a ratio of 1:100 and 1:1000 (equivalent to a dilution of the virus suspension of 10^{-3} and 10^{-4} in the disinfectant test batch) with medium without foetal calf serum. Then they were added to the tissue culture, as with the batches for testing the disinfectant action, for 1 h and then drawn off. After incubation for 2 days at 37°C , staining was used to test whether the cell lawn had been damaged by the disinfectant.

As an indication of the resistance of the test virus and for comparability with other studies, a "formaldehyde control" was carried out. For this, 1 part of the virus suspension was mixed with 4 parts of phosphate-buffered saline (0.1 M; pH 7; "Dulbecco's PBS") and the entire volume was added to a formalin solution containing 1.4 g of formaldehyde in 100 ml of solution (final concentration: 0.7 g of $\text{HCHO}/100\text{ ml}$). After 5, 15 and 60 min of exposure time, the action of formaldehyde was stopped, as with the disinfectant test, by diluting to 1:100 and the remaining infectivity of the poliovirus was determined in the plaque test in further serial ten-fold dilutions.

Results:

Control experiments:

The "virus control", in the batch with twice-distilled water, gave a virus concentration of $1.6 \cdot 10^3$ infectious units/ml, in the batch containing serum albumin, $1.2 \cdot 10^3$ infectious units/ml, and in the batch containing foetal calf serum $1.0 \cdot 10^3$ infectious units/ml. The "toxicity control", after dilution of the test batch to 1:100 (equivalent to a dilution of the virus suspension of 10^{-3}) showed slight damage of the cell lawn. At a dilution of 1:1000, toxicity was no longer observable. Thus under the test conditions, a decrease in virus concentration under the action of disinfectant can be followed to a virus concentration of $5 \cdot 10^3$ infectious units/ml in the virus suspension (in both wells of the dilution 10^{-4} , plaque is then no longer visible) and at a starting concentration of at least 10^8 infectious virus particles/ml, a decrease in virus concentration over at least 4.5 powers of ten is observable. Since the test guideline for detecting the effectiveness of a disinfectant only requires a decrease in virus concentration by at least 4 powers of ten, compliance with this condition can be detected using the experimental batch chosen.

In the batch containing 0.7% strength formaldehyde, after an exposure time of 5 min, a virus concentration of $1.05 \cdot 10^6$ /ml was measured, after 15 min $1 \cdot 10^3$ /ml, and after 60 min $\leq 5 \cdot 10^2$ /ml. These are expected values which confirm the results of earlier experiments: 0.7% strength formalin is usually able to reduce the concentration of poliovirus by more than 4 powers of ten within 30 min.

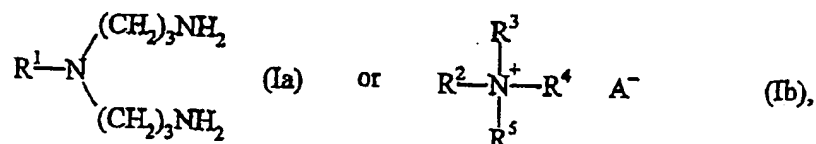
Effectiveness of the disinfectants against poliovirus:

After 30, 60 and 120 min exposure times of 6% strength dilution of the disinfectant composition from Example 20, in the batch containing foetal calf serum at the virus dilution 10^{-4} , in each of the two test wells plaque was no longer observed. Thus after the disinfectant treatment, a virus concentration of $\leq 5 \cdot 10^3$

infectious units/ml was present. This result was found not only with low protein load (batch with twice-distilled water), but also with medium (batch containing 2% strength serum albumin) and high protein load (batch containing foetal calf serum). Thus, compared with the control determination without disinfectant, there was a decrease in virus concentration by at least $4.5 \log_{10}$ or powers of ten. Thus the condition for effectiveness for registration as instrument disinfectant in the Federal Republic of Germany is fulfilled.

Claims

1. Use of a disinfectant composition comprising
a) an amine and/or quaternary ammonium salt of the
general formula



where R^1 is C_{6-18} -alkyl

R^2 is benzyl or C_{6-18} -alkyl

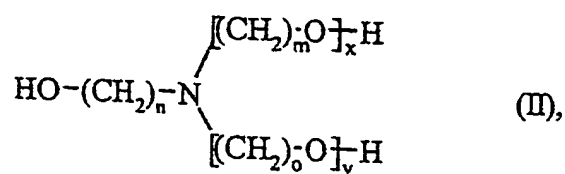
R^3 is C_{1-18} -alkyl or $-(\text{CH}_2)_2-\text{O}-\text{R}^6$ where $n = 1-20$

R^4 and R^5 independently of one another are C_{1-4} -alkyl

R^6 is hydrogen or unsubstituted or substituted phenyl

and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and

- b) at least one alkanolamine of the general formula



where n and, if present, m and o independently of one another have the value 2 or 3

and x and y independently of one another have the value 0 or 1, or a corresponding salt;

in the mass ratio I:II of 20:1 to 1:20 as virucidal agent.

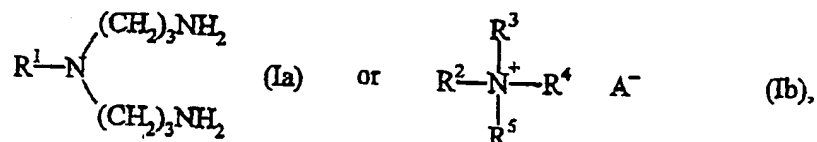
2. Use according to Claim 1, characterized in that the amine or quaternary ammonium salt is selected from the group consisting of N,N -bis-

- (3-aminopropyl)dodecylamine, *N,N*-bis(3-amino-propyl)octylamine, didecyldimethylammonium salts, dioctyldimethylammonium salts, octyldecyldimethylammonium salts, coconutalkyldimethylbenzylammonium salts and benzyldimethyloxethylammonium salts and mixtures of these compounds.
- 5
3. Use according to Claim 1 or 2, characterized in that the alkanolamine (II) is selected from the group consisting of monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol.
- 10
4. Use according to one of Claims 1 to 3, characterized in that the mass ratio I:II is between 1:5 and 5:1.
- 15
5. Use according to one of Claims 1 to 4, characterized in that the disinfectant composition comprises water as solvent.
- 20
6. Use according to one of Claims 1 to 5, characterized in that the disinfectant composition additionally comprises one or more aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.
- 25
7. Use according to one of Claims 1 to 6 in surface disinfection and instrument disinfection.
- 30
8. Use according to one of Claims 1 to 6 in laundry disinfection.
- 35
9. Use according to one of Claims 1 to 6 in hand disinfection.
10. Use according to one of Claims 1 to 6 in chemical toilets.

Abstract

A description is given of the use of disinfectant compositions comprising

- a) at least one amine and/or quaternary ammonium salt of the general formula



where R^1 is C_{6-18} -alkyl

R^2 is benzyl or C_{6-18} -alkyl

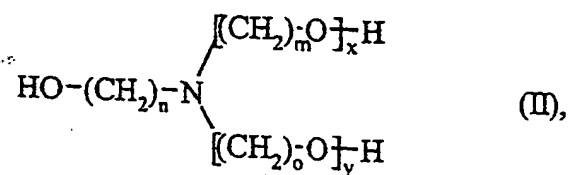
R^3 is C_{1-18} -alkyl or $-(\text{CH}_2)_2\text{-O}]_n\text{R}^6$ where $n = 1-20$

R^4 and R^5 independently of one another are C_{1-4} -alkyl

R^6 is hydrogen or unsubstituted or substituted phenyl

and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and

- b) at least one alkanolamine of the general formula



where n and, if present, m and o independently of one another have the value 2 or 3


and x and y independently of one another have the value 0 or 1, or a corresponding salt;

in the mass ratio I:II of 20:1 to 1:20 as virucidal agent, in particular towards polioviruses. The compositions are also distinguished by good bactericidal, and in particular also fungicidal,

effectiveness, even at low usage concentrations.

Basle, 18 January 2002

Dr N. Riegler

A handwritten signature in black ink, consisting of a large, stylized 'R' followed by a series of loops and a final vertical stroke.

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